A/RE

EXPRESS MAIL NO. EK044771881US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING REISSUE PATENT APPLICATION

The Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

This is a request for a reissue patent application of:

Inventor: Paul R. Burnett, John E. Van Hamont, Robert H. Reid, Jean A. Setterstrom, Thomas C. Van Cott, Deborah L. Birx

Title: VACCINES AGAINST INTRACELLULAR PATHOGENS USING ANTIGENS ENCAPSULATED WITHIN BIODEGRADABLE-BIOCOMPATIBLE MICROSPHERES

Patent No: 5,762,965 **Issue Date:** June 9, 1998

Serial No.: 08/598,874 **Filing Date:** February 9, 1996

Date of this reissue application filing: June 2, 2000

Attorney docket no: Army 105

This application for reissue includes:

_X_ Specification: 8 pages (only spec. and claims)
_X_ Abstract: 1 page(s)
_X__ Drawings:* 2 sheet(s) per set:
_1__ set(s) informal (Figs. 1 & 2);
___ set(s) formal of size ___A4 ___ 13" ___ 14"
***see separate letter requesting transfer of the drawings from the patent file**
_X_ Declaration
_X_ Assent of Assignee
___ Request for Title Search
___ Verified Statement establishing small entity status
_X_ Power of Attorney (included in Declaration)
_X_ Also attached:
(1) Form PTO-1449
(2) copies of references cited
(3) Letter Requesting Transfer of Drawings from the Patent File

(4) Amendment to Reissue Application under 37 CFR §1.121 & 1.173
(5) 2 pages of additional claims 15-33 (pages 7 and 8)

Priority is claimed under 37 CFR 1.78(a)(3) based on: continuation in part of Ser. No. 242,960, filed May 16, 1994 and Ser. No. 08/446,149, filed May 22, 1995, which is a continuation of Ser. No 590,308, filed March 16, 1984, abandoned, said Ser. No. 242,960, is a continuation-in-part of Ser. No. 867,301, files April 10, 1992, Patent No. 5,417,986, which is a continuation-in-part of Ser. No. 805,721, filed November 21, 1991, abandoned, which is a continuation-in-part of Ser. No. 690,485, filed April 24, 1991, abandoned, which is a continuation-in-part of Ser. No. 521,945, filed May 11, 1990, abandoned.

Fee Calculation:

Basic Filing Fee ($760/$380)				$ 690
Claim fees:	Claims in reissue app.	Claims in orig. patent		
Total effective claims	(A) 44	(B) 25	(A) minus [larger of (B) or 20] = 19 x $18/$9=	$342
Total indep. claims	(D) 2	(E) 1	(D) minus (E) = 1 x $78/$39=	$78
Title Search Fee ($25.00)				$
Petition Fee per Rule 17(h) for Rule 47 Petition ($130.00)				$
Other Fees:				$

TOTAL FEES $1110.00

CHARGE STATEMENT: The Commissioner is hereby authorized to charge the above fee and any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Deposit Account No. 21-0380, for which purpose a duplicate copy of this sheet is attached.

Submitted by: Caroline Nash

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NOTE: File in duplicate with post card receipt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF
Burnett, et al.

REISSUE of U. S. Patent No. 5,762,965

Appln. No.: TBA

Group Art Unit: TBA

Filed: Herewith

Examiner: TBA

Title: VACCINES AGAINST INTRACELLULAR PATHOGENS USING ANTIGENS ENCAPSULATED WITHIN BIODEGRADABLE-BIOCOMPATIBLE MICROSPHERES

ASSENT OF ASSIGNEE

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

The undersigned states the following:

1. The undersigned verifies that he is empowered to sign this statement on behalf of the Assignee, the Government of the Unites States, as represented by the Secretary of the Army.

2. The Government of the United States, as represented by the Secretary of the Army, is the Assignee of the entire interest in the United States Letters Patent No. 5,762,965, as indicated in Assignment recorded on , Reel # 008353, Frame # 0262, recorded on February 18, 1997.

3. The evidentiary documents regarding the Assignment of the United States Letters Patent No. 5,762,965 have been reviewed and the undersigned Assignee certifies that, to the best of Assignee's knowledge and belief, title to this patent is in the Assignee.

4. The undersigned Assignee hereby assents to the accompanying reissue application.

The United States of America

By: X _____

Martin H. Crumrine
Colonel, MS
Commander

Date: X 14 APR 00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF
Burnett, et al.

REISSUE of U. S. Patent No. 5,762,965

Appln. No.: TBA | Group Art Unit: TBA

Filed: Herewith | Examiner: TBA

Title: VACCINES AGAINST INTRACELLULAR PATHOGENS USING ANTIGENS ENCAPSULATED WITHIN BIODEGRADABLE-BIOCOMPATIBLE MICROSPHERES

AMENDMENT TO REISSUE APPLICATION
UNDER 37 CFR §1.121and 37 CFR §1.173

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Please consider the following amendments to the specification and claims of this reissue application made pursuant to 37 CFR §1.121 and 37 CFR §1.173. Applicants respectfully request entry thereof.

IN THE INVENTORS:

Replace the last mispelled inventors name with the following:

[Debrah L. Birx] Deborah Birx

IN THE SPECIFICATION:

Replace the figure descriptions beginning at column 2, line 13 with the following:

FIG. 2 indicates [control] cytotoxic T lymphocyte induction in mice immunized with rgp 160; and

Replace the paragraph beginning at column 2, line 19 with the following:

This invention relates to a novel pharmaceutical composition , a microcapsule/sphere formulation, which comprises an antigen encapsulated within a biodegradable polymeric matrix, such as poly(DL-lactide co glycolide) (PLG), wherein the molecular weight of the PLG is about 4,000 to 100,000 daltons and wherein the relative ratio between the lactide and glycolide component of the PLG is within the range of 52:48 to 0:100, and its use, as a vaccine, in the effective induction of antiviral immune responses comprising both virus specific cytotoxic T lymphocytes and antibodies reactive against native viral antigens. In the practice of this invention, applicants found that when a complex (oligomeric) native envelope protein of HIV-1 was encapsulated in PLG microspheres, it retained its native antigenicity and function upon its release in vitro. Furthermore, when used as a vaccine in animals, this product elicited HIV-specific cytotoxic T hymphocytes and antibodies reactive with native (oligomeric) HIV-1 envelope protein.

Replace the paragraph beginning at column 2, lines 46 with the following:

Microencapsulation of immunogens: PLG microspheres ranging from 1nanometer to 20μm in diameter and containing a 0.5 to 1.0 antigen core load were prepared by a solvent extractive method. 0.5 to 5.0% by weight antigen core load could also be used. The solvent extraction method involves dissolving the viral antigen and sucrose (1:4 ration w:w) in 1 ml of deionized water. This solution is flash frozen and lyophilized. The resulting antigen-loaded sucrose particles are resuspended in acetonitrile and mixed

in PLG copolymer dissolved in acetonitrile. This antigen-polymer mixture is then emulsifyed into heavy mineral oil, transferred into heptane and mixed for 30 min to extract the oil and acetonitrile from the nascent spheres. The spheres are harvested by centrifugation, washed three times in heptane and dried overnight under vacuum. Microsphere size was determined by both light and scanning electron microspopy. The antigen core load was determined by quantitative amino acid analysis of the microspheres following complete hydrolysis in 6N hydrochloric acid.

Replace the paragraph beginning at column 5, line 1 with the following:

antigen to denatured gp 120 [(FIGS. 2, 3, and 4)] (FIG. 2, nos. 3 and 4) and the preferred binding of antibodies elicited by microspheres loaded with native (oligomeric) antigen to native gp 120 [(FIGS. 2, 7-8)] (FIG 2, nos. 7 and 8).

IN THE CLAIMS:

Amend claim 7 as follows:

Claim 7. (Amended) A vaccine [consisting of] comprising a blend of the immunostimulating compositions of claim 5 [described in claims 5 or 6].

Amend claim 8 as follows:

Claim 8. (Amended) The immunostimulating composition described in claim 5, employed as a [parentally] parenterally administered vaccine wherein the diameter size range of said vaccine microspheres lies between 1 nanometer and 20 microns.

Amend claim 11 as follows:

Claim 11. (Amended) A vaccine [consisting of] comprising a blend of the immunostimulating compositions of claim 6 [described in claims 5 or 6].

Amend claim 12 as follows:

Claim 12. (Amended) The immunostimulating composition described in claim 6, employed as a [parentally] parenterally administered vaccine wherein the diameter size range of said vaccine microspheres lies between 1 nanometer and 20 microns.

Amend claim 13 as follows:

Claim 12. (Amended) The immunostimulating composition described in claim 7, employed as a [parentally] parenterally administered vaccine wherein the diameter size range of said vaccine microspheres lies between 1 nanometer and 20 microns.

Please add claims 15-33 as follows:

Claim 15. An immunostimulating composition comprising encapsulating microspheres, wherein said encapsulating microspheres comprise:

a biodegradable-biocompatible poly(DL-lactide-co-glycolide) as a bulk matrix and

an immunogenic substance comprising a conformationally native subunit of chronic intracellular pathogen which, in the course of natural infection with that pathogen, is exposed to the host immune system on the surface of free pathogen and/or pathogen-infected cells.

Claim 16. The immunostimulating composition of claim 15, wherein the encapsulating microspheres are produced by a solvent extraction process.

Claim 17. The immunostimulating composition of claim 15, wherein the encapsulating microspheres are produced by a solvent evaporation process.

Claim 18. The immunostimulating composition of claim 15, wherein the antigen is pre-encapsulated into a conformationally stabilizing hydrophilic matrix comprising an appropriate mono, di- or tri-saccharide or other carbohydrate substance by lyophilization prior to its final encapsulation into the PLG microsphere.

Claim 19. The immunostimulating composition of claim 18, wherein the encapsulating microspheres are produced by a solvent extraction process.

Claim 20. The immunostimulating composition of claim 19, wherein said solvent extraction process employs acetonitrile as the polymer solvent, mineral oil as the emulsion's external phase, and heptane as the extractant.

Claim 21. The immunostimulating composition of claim 15, wherein said microspheres further comprise a pharmaceutically acceptable adjuvant.

Claim 22. The immunostimulating composition of claim 15, wherein a molecular weight of the poly(DL-lactide-co-glycolide) is 4,000 to 100,000 daltons.

Claim 23. The immunostimulating composition of claim 15, wherein the relative ratio between the amount of the lactide:glycolide components of the matrix is within the range of 52:48 to 0:100.

Claim 24. The immunostimulating composition of claim 15, wherein the immunogenic substance is a native (oligomeric)HIV-1 envelope antigen.

Claim 25. The immunostimulating composition of claim 15, wherein the amount of said immunogenic substance with in the microsphere comprises between 0.5% to 5.0% of the weight of said composition.

Claim 26. The immunostimulating composition of claim 15, wherein the diameter size range of the microspheres is between 0.1 - 20μm.

Claim 27. The immunostimulating composition of claim 15, wherein the size of more than 50% (by volume) of the vaccine microspheres is between 5 and 10 μm in diameter.

Claim 28. The immunostimulating composition of claim 15, wherein said immunostimulating composition is administered as a mucosal vaccine or a parenteral vaccine.

Claim 29. The immunostimulating composition of claim 28 in the form of a mucosal administerable vaccine wherein the diameter size range of the vaccine microspheres is between 5-10μm.

Claim 30. The immunostimulating composition of claim 28 in the form of a parenteral administerable vaccine wherein the diameter size range of the vaccine microspheres is between 0.1 - 20μm.

Claim 31. The immunostimulating composition of claim 30, wherein said immunogenic substance is present in an amount of 0.5 - 5% antigen by weight.

Claim 32. The immunostimulating composition of claim 15, wherein said bulk matrix encapsulates said immunogenic substance protectively and/or facilitates its interaction with the host immune system to augment its immunogenicity.

Claim 33. A vaccine comprising the immunostimulating composition of claim 15.

REMARKS

The specification has been amended at column 5 to correct typographical errors. Support for the amendment to column 5 is found in the figures.

The spelling of the inventor, Deborah Birx has been corrected.

The figure description in column 2 beginning at line 13 of FIG. 1 has been amended to correct an inadvertent inaccuracy. Support for this amendment is found in the Figure 1 and in column 2, line 27.

The specification has been amended at column 2, in the paragraph beginning at line 19 to include the molecular weight range of the polymer. Support for this amendment is found in the originally submitted claims 1 and 6.

The specification has been amended at column 2, in the paragraph beginning at line 46 to insert ---1 nanometer --- to indicate the lower range of microsphere diameter and to indicate an amount of immunogenic substance of 0.5% to 5.0% of the weight of the composition. Support for these amendments is found in the originally submitted claims 4 and 8.

Claims 7 and 11 have been amended to correct improper multiple dependencies and to change the term "consisting of" to --- comprising ---. Claims 8, 12 and 13 have been amended to correct spelling errors.

New claims 15-33 are supported by claims 1-14 and the description as originally filed. No new matter has been added.

A marked up specification showing insertions with underlining and deletions with bracketing is submitted herewith in accordance with 37 CFR §1.173.

Respectfully submitted:

Date: June 2, 2000 By: Caroline Nash

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United States Patent [19]

Burnett et al.

[54] **VACCINES AGAINST INTRACELLULAR PATHOGENS USING ANTIGENS ENCAPSULATED WITHIN BIODEGRADBLE-BIOCOMPATIBLE MICROSPHERES**

[75] Inventors: **Paul R. Burnett**, Silver Spring; **John E. Van Hamont**, Ft. Meade; **Robert H. Reid**, Kensington, all of Md.; **Jean A. Setterstrom**, Alpharetta, Ga.; **Thomas C. Van Cott**, Brookeville; [Debrah L.]Deborah L. **Birx**, Potomac, both of Md.

[73] Assignee: **The United States of America as represented by the Secretary of the Army**, Washington, D.C.

[21] Appl. No.: **598,874**

[22] Filed: **Feb. 9, 1996**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 242,960, May 16, 1994, and Ser. No. 446,149, May 22, 1995, which is a continuation of Ser. No. 590,308, Mar. 16, 1984, abandoned, said Ser. No. 242,960, is a continuation-in-part of Ser. No. 867,301, Apr. 10, 1992, Pat. No. 5,417,986, which is a continuation-in-part of Ser. No. 805,721, Nov. 21, 1991, abandoned, which is a

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[11] Patent Number: 5,762,965

[45] Date of Patent: Jun. 9, 1998

continuation-in-part of Ser. No. 690,485, Apr. 24, 1991, abandoned, which is a continuation-in-part of Ser. No. 521,945, May 11, 1990, abandoned.

[51] **Int. Cl.**[6] **A61K 9/00**; A61K 9/66; A61K 9/14; A61F 13/00

[52] **U.S. Cl.** **424/499**; 424/426; 424/455; 424/486; 424/488; 424/422

[58] **Field of Search** 424/499, 426, 424/455, 486, 488, 422

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,863,735	9/1989	Kohn et al.	424/422
4,897,268	1/1990	Tice et al.	424/422

Primary Examiner—Theodore J. Criares
Attorney, Agent, or Firm—Werten F. W. Bellamy

[57] **ABSTRACT**

This invention relates to parenteral and mucosal vaccines against diseases caused by intracellular pathogens using antigens encapsulated within a biodegradable-biocompatible microspheres(matrix).

14 Claims, 2 Drawing Sheets

VACCINES AGAINST INTRACELLULAR PATHOGENS USING ANTIGENS ENCAPSULATED WITHIN BIODEGRADBLE-BIOCOMPATIBLE MICROSPHERES

II. CROSS REFERENCE

This application is a continuation-in-part of U.S. patent application Ser. No. 08/242,960, filed May 16, 1994, pending; which in turn is a continuation-in-part of U.S. patent application Ser. No. 07/867,301 filed Apr. 10, 1992, now U.S. Pat. No. 5,417,986 which in turn is a continuation-in-part of U.S. patent application Ser. No. 07/805,721 filed Nov. 21, 1991; now abandoned, which in turn is a continuation-in-part of U.S. patent application Ser. No. 07/690,485 filed Apr. 24, 1991, now abandoned; which in turn is a continuation-in-part of U.S. patent application Ser. No. 07/521,945 filed May 11, 1990, now abandoned. Additionally, this application is a continuation-in-part of U.S. patent application Ser. No. 08/446,149 filed May 22, 1995, pending; which in turn is a continuation of U.S. patent application Ser. No. 06,590,308 filed Mar. 16, 1984, now abandoned.

I. GOVERNMENT INTEREST

The invention described herein may be manufactured, licensed and used by or for governmental purposes without the payment of any royalties to us thereon.

III. FIELD OF THE INVENTION

This invention relates to parenteral and mucosal vaccines against diseases caused by intracellular pathogens using antigens encapsulated within biodegradable-biocompatible microspheres(matrix).

IV. BACKGROUND OF THE INVENTION

Most infections by viruses and other intracellular pathogens are countered in the human host by a combination of humoral (antibody) and cellular (lymphocyte and phagocyte) immune effectors. Although the precise identity of immune effectors capable of protecting the host against some chronic intracellular pathogens (e.g. HIV-1) remains unknown, attempts to develop preventive and therapeutic vaccines still focus on the induction of appropriate humoral and cellular immune responses. Furthermore, since most human viral pathogens (including HIV-1) are transmitted across mucosal surfaces, it is important that vaccines induce such responses locally (at the mucosal surface) as well as systemically and that they be durable for long-term protection.

The issues of durability and mucosal immunogenicity have been previously addressed by encapsulating vaccine antigens in appropriately-sized biodegradable, biocompatible microspheres made of lactide/glycolide copolymer (the same materials used in resorbable sutures). It has been shown that such microspheres can be made to release their load in a controlled manner over a prolonged period of time and can facilitate the interaction of their contents with the local immune system when administered mucosally.

In the case of HIV-1 infection, there is insufficient information at this time regarding the virus and its interactions with the human immune system to permit the rational design of a preventive vaccine. However, it has been noted that many candidate HIV vaccines tested to date fail to elicit antibodies capable of neutralizing wild-type HIV-1 or binding to native HIV-1 proteins, fail to induce a substantial

population of effector cells capable of destroying HIV-1-infected cells, and fail to induce significant responses at mucosal surfaces. A possible approach to overcoming these problems (applicable to both HIV-1 and other chronic intracellular pathogens) is to identify a native protein, accessible to the immune system on the surface of both free virus and infected cells, and present it to the immune system (systemic and mucosal) encapsulated in microspheres to protect and augment its immunogenicity.

V. DESCRIPTION OF DRAWINGS

FIG. 1 indicates [control] cytotoxic T lymphocyte in mice immunized with rgp 160; and

FIG. 2 indicates "Native"/denatured rgp 120 (IIIB) Binding Ratios.

V. DESCRIPTION OF THE INVENTION

This invention relates to a novel pharmaceutical composition, a microcapsule/sphere formulation, which comprises an antigen encapsulated within a biodegradable polymeric matrix, such as poly(DL-lactide co glycolide) (PLG), wherein the molecular weight of the PLG is about 4,000 to 100,000 daltons and wherein the relative ratio between the lactide and glycolide component of the PLG is within the range of 52:48 to 0:100, and its use, as a vaccine, in the effective induction of antiviral immune responses comprising both virus-specific cytotoxic T lymphocytes and antibodies reactive against native viral antigens. In the practice of this invention, applicants found that when a complex (oligomeric) native envelope protein of HIV-1 was encapsulated in PLG microspheres, it retained its native antigenicity and function upon its release in vitro. Furthermore, when used as a vaccine in animals, this product elicited HIV-specific cytotoxic T lymphocytes and antibodies reactive with native (oligomeric) HIV-1 envelope protein.

The following examples illustrate the invention:

EXAMPLE 1

Materials and Methods

Immunogens. Non-CD4-binding, baculo-expressed, recombinant gp 160_{IIIB} (rgp 160) was obtained from MicroGeneSys (Meriden, Conn.). CD4-binding, oligomeric gp 160 CDC451 (o-gp 160) was obtained from Advanced BioScience Laboratories (Kensington, Md.).

Microencapsulation of immunogens: PLG microspheres ranging from 1 nanometer to 20 um in diameter and containing a 0.5 to 1.0% antigen core load were prepared by a solvent extractive method. 0.5 to 5.0% by weight antigen core load could also be used. The solvent extraction method involves dissolving the viral antigen and sucrose (1:4 ratio w:w) in 1 ml of deionized water. This solution is flash frozen and lyophilized. The resulting antigen-loaded sucrose particles are resuspended in acetonitrile and mixed into PLG copolymer dissolved in acetonitrile. This antigen-polymer mixture is then emulsifyed into heavy mineral oil, transferred into heptane and mixed for 30 min to extract the oil and acetonitrile from the nascent spheres. The spheres are harvested by centrifugation, washed three times in heptane and dried overnight under vacuum. Microsphere size was determined by both light and scanning electron microscopy. The antigen core load was determined by quantitative amino acid analysis of the microspheres following complete hydrolysis in 6N hydrochloric acid.

Analysis of immunogen spontaneously released from microspheres in vitro by binding to soluble CD4 and recognition by HIV-positive patient serum. PLG microspheres loaded with native (oligomeric) gp 160 were suspended in

phosphate-buffered saline, pH 7.4 (PBS), incubated at 37 C. for 3 h, and then at 4 C overnight. The microspheres were then sedimented by centrifugation (2 min at 200×g), the supernatants harvested, and the released gp 160 assayed for binding to CD4 and recognition by HIV-positive patient serum by surface plasmon resonance (described below). A sample of the stock protein used for microencapsulation was assayed for comparison.

Immunization of animals. HIV-seronegative, 8–10 week old NZW rabbits were immunized intramuscularly with rgp 160- or o-gp 160-loaded PLG microspheres suspended in PBS or with alum-adjuvanted rgp 160 in PBS. Groups receiving rgp 160-loaded microspheres (n=2) were primed with 50 ug of immunogen on day 0 and boosted with 25 ug on day 42. Groups receiving o-gp 160-loaded microspheres (n=3) were primed with 70 ug of immunogen on day 0 and boosted with 35 ug on day 56. Groups receiving alum-adjuvanted rgp 160 (n=2) got 85 ug of immunogen on days 0, 7, and 28.

BALB/c mice were immunized subcutaneously with rgp 160-loaded PLG microspheres suspended in PBS or with alum-adjuvanted rgp 160 in PBS. The mice in all groups (n=4) received 10 ug of immunogen on days 0 and 21.

Determination of the ratio of antibody binding to "native"/denatured rgp 120_{IIIB} measured by surface plasmon resonance (SPR). Real-time binding interactions between ligand (gp 120 covalently linked to a biosensor matrix) and ligate (Abs in solution) were measured using surface plasmon resonance (BIAcore, Pharmacia Biosensor, Piscataway, N.J.). "Native"rgp 120(IIIB) (Genentech, South San Francisco, Calif.) or reduced, carboxymethylated (denatured) rgp 120(IIIB) (Genentech) was covalently linked to the biosensor dextran matrix. Sera and mAbs were diluted in HBS running buffer (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.05% (BIAcore) surfactant P20, pH 7.4) and injected through the dextran matrices at a flow rate of 5 ul/min. The binding value of each serum or mAb was measured in resonance units (RU), and the "native"/denatured gp 120 ratios were determined by dividing the corresponding RU values and correcting for small differences in matrix concentration. Controls included an HIV-positive patient serum and mAb 1c1.

Assessment of HIV-specific cell-mediated immunity in immunized mice by secondary CTL assay. The spleens of BALB/c mice immunized on days 0 and 21 were harvested and single cell suspensions prepared aseptically in complete RPMI medium on day 35. The cells were then pooled within experimental groups (n=4), underlay with ficoll, centrifuged 30 min at 450×g (RT), washed, and resuspended in complete RPMI medium. Following a 1 h stimulation with peptide p18 (1 uM) at 37° C., the cell suspensions were diluted with complete RPMI supplemented with 2ME (1:1000) and transferred to flasks for a 6 day incubation at 37° C. After 2 days, recombinant IL-2 (10 u/ml) was added to all flasks. On day 6, P815 target cells were pulsed with peptide p18 (1 uM) or with nothing (control) in PBS supplemented with 0.1% BSA. $3\times10^{\wedge}6$ target cells were labelled with 300 uCi of ^{51}Cr, washed, and plated out with the effector cells at effector:target (E:T) ratios of 45:1, 15:1, 5:1, and 1.7:1. After a 6 h incubation at 37° C., the supernatants were harvested and counted, and % specific lysis was calculated.

Results

Comparison of the native (oligomeric) gp 160 prior to microencapsulation and following spontaneous release from PLG microspheres showed the two to be essentially indistinguishable in terms of their binding to CD4 and recogni-

tion by HIV-positive patient serum. (Table 1). This retention of conformation-dependent binding shows that structure of the antigen is not appreciably altered by the microencapsulation process.

FIG. 1 shows the data from a cytotoxic T lymphocyte (CTL) assay performed on the speen cells of mice which had had been previously immunized with either HIV-1 envelope protein encapsulated in PLG microspheres (dark squares) or the same protein administered in a conventional way with alum adjuvant (dark diamonds). These data indicate that microencapsulation of HIV-1 envelope protein in PLG microspheres results in a vaccine that induces significantly greater anti-HIV CTL activity than does alum-adjuvanted vaccine. The open symbol groups represent controls run to assure that the activity being measured is virus-specific.

FIG. 2 shows the results of an assay designed to measure the relative binding of antibodies to native vs denatured viral protein. These data show that rabbits immunized with a non-native HIV-1 protein encapsulated in PLG (#5 and 6) develop antibodies which show greater binding to denatured (vs native) protein (indicated by a ratio<1). On the other hand, rabbits immunized with a native HIV-1 protein encapsulated in PLG microspheres (#10–12) develop antibodies which show greater binding to native viral protein (indicated by ratio>1). This retention of each proteins antigenicity constitutes an additional piece of evidence that the structure of antigens loaded in PLG microspheres are preserved.

EXAMPLE 2

Materials and Methods

This experiment was similar to that described in Example 1 except for the method of microencapsulation employed.

Microencapsulation of immunogens: PLG microspheres ranging from 1 to 15 um in diameter and containing a 0.5 to 1.0% antigen core load were prepared by a solvent evaporation method. The solvent evaporation method involves emulsifying the viral antigen dissolved in deionized water into poly(DL-lactide-co-glycolide) polymer dissolved in methylene chloride. This emulsion is mixed into 0.9% polyvinyl alcohol and stirred. After 10 min of stirring, 0.35 l of water is added and gentle mixing is continued for 1.5 h. The resulting spheres are harvested by centrifugation, washed three times in distilled water, and dried overnight under vacuum. Microsphere size was determined by both light and scanning electron microscopy. The antigen core load was determined by quantitative amino acid analysis of the microspheres following complete hydrolysis in 6N hydrochloric acid.

Results

Analysis of spontaneously released antigen showed it to retain its CD4 binding capacity. Its native antigenicity (recognition by the serum of an HIV-positive patient) was only slightly less than that of the antigen prior to encapsulation and following spontaneous release from microspheres produced by a solvent extraction method (Table 1).

The results of immunizing animals with either non-native (denatured) or native oligomeric gp 160 in PLG microspheres produced by a solvent evaporation method were essentially indistinguishable from those obtained using microspheres produced by a solvent extraction method (example 1). Microencapsulated antigen induced significantly greater CTL activity than antigen administered in a conventional alum-adjuvanted formulation. Furthermore, preservation of the structure of PLG-microencapsulated antigens is supported by the findings of preferential binding of antibodies elicited by microspheres loaded with denatured

antigen to denatured gp 120 [(FIGS. 2, 3, and 4)] (FIG. 2, nos. 3 and 4) and the preferred binding of antibodies elicited by microspheres loaded with native (oligomeric) antigen to native gp 120 [(FIGS. 2, 7-8)] (FIG 2, nos. 7 and 8).

TABLE 1

BIA (released o-gp160)
Capture o-gp160-451 (stock vs microsphere-released)
on tvc 391 fc3/fc4 sCD4 (4 mg/m)
1 ul/min flow rate for o-gp160 inj.; 5 ul/min for all others

Ligate	RU	HIV+/sCD4 (RU ratio)
gp120-MN 1:10	3286	
HIV+ 1:100	54	
NHS 1:100	3	
HIV+ pool 1:100	47	
o-gp160 (tvc281)	1772	
HIV+	3259	1.84
tvc281	1848	
NHS	−36	
tvc281	1762	
HIV+ pool	2597	1.47
tvc281-PLG-EV	3342	
HIV+	4594	1.37
tvc281	3222	
NHS	7	
tvc281	3210	
HIV+ pool	3336	1.04
tvc281-PLG-EX	1855	
HIV+	3760	2.04
tvc281	1839	
NHS	2	
tvc281	1850	
HIV+ pool	2745	1.48
gp120-MN 1:10	2914	
HIV+ 1:100	14	
NHS 1:100	−2	
HIV+ pool 1:100	14	
tvc281	1099	
HIV+	1083	0.99
tvc281	1022	
HIV+ pool	1395	1.36
tvc281-PLG-EV	1595	
HIV+	1322	0.83
tvc281	1535	
HIV+ pool	1781	1.16

In view of the above it will be seen that the objects of the invention are achieved. As various changes could be made in the above materials and methods without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not limiting.

We claim:

1. An immunostimulating composition comprising encapsulating microspheres comprised of (a) a biodegradable-biocompatible poly(DL-lactide-co-glycolideas the bulk matrix produced by a solvent evaporation process wherein the molecular weight of the copolymer is between 4,000 to 100,000 daltons and (b) an immunogenic substance consisting of a conformationally native subunit of chronic intracellular pathogen which, in the course of natural infection with that pathogen, is exposed to the host immune system on the surface of free pathogen and/or pathogen-infected cells.

2. The immunostimulating composition described in claim 1 wherein the antigen is pre-encapsulated into a conformationally stabilizing hydrophilic matrix consisting of an appropriate mono, di- or tri-saccharide or other carbohydrate susbstance by lyophilization prior to its final encapsulation into the PLG microsphere by a solvent extraction process employing acetonitrile as the polymer solvent, mineral oil as the emulsion's external phase, and heptane as the extractant.

3. The immunostimulating compositions described in claims 1 or 2 wherein the immunogenic substance is a native (oligomeric)HIV-1 envelope antigen that is conformationally stabilized by the polymer matrix and serves to elicit in animals the production of HIV specific cytotoxic T lumphocytes and antibodies preferentially reactive against native HIV-1 envelope antigen.

4. The immunostimulating compositions described in claim 3 wherein the amount of said immunogenic substance within the microcapsule comprises between 0.5% to 5.0% of the weight of said composition.

5. The immunostimulating compositions describe in claim 4 wherein the relative ratio between the amount of the lactide:glycolide components of said matrix is within the range of 52:48 to 0:100.

6. The immunostimulating compositions described in claim 5 wherein the molecular weight of said copolymer is between 4,000 to 50,000 daltons.

7. A vaccine [consisting of] comprising a blend of the immunostimulating compositions of claim 5 [described in claims 5 or 6].

8. The immunostimulating compositions described in claim 5, employed as a parenterally [parentally] administered vaccine wherein the diameter size range of said vaccine microspheres lies between 1 nanometer and 20 microns.

9. The immunostimulating compositions described in claim 5, employed as a mucosal vaccine wherein the size of more than 50% (by volume) of said vaccine microspheres is between 5 to 10 microns in diameter.

10. A composition in accordance with claim 1 wherein the microspheres further contain a pharmaceutically-acceptable adjuvant.

11. A vaccine [consisting of] comprising a blend of the immunostimulating compositions of claim 6 [described in claims 5 or 6]

12. The immunostimulating compositions described in claim 6 employed as a parenterally [parentally] administered vaccine wherein the diameter size range of said vaccine microspheres lies between 1 nanometer and 20 microns.

13. The immunostimulating compositions described in claim 7 employed as a parenterally [parentally] administered vaccine wherein the diameter size range of said vaccine microspheres lies between 1 nanometer and 20 microns.

14. The immunostimulating compositions described in claim 6 employed as a mucosal vaccine wherein the size of more than 50% (by volume) of said vaccine microspheres is between 5 to 10 microns in diameter.

* * * * *

Please add claims 15-33 as follows:

Claim 15. An immunostimulating composition comprising encapsulating microspheres, wherein said encapsulating microspheres comprise:

a biodegradable-biocompatible poly(DL-lactide-co-glycolide) as a bulk matrix and

an immunogenic substance comprising a conformationally native subunit of chronic intracellular pathogen which, in the course of natural infection with that pathogen, is exposed to the host immune system on the surface of free pathogen and/or pathogen-infected cells.

Claim 16. The immunostimulating composition of claim 15, wherein the encapsulating microspheres are produced by a solvent extraction process.

Claim 17. The immunostimulating composition of claim 15, wherein the encapsulating microspheres are produced by a solvent evaporation process.

Claim 18. The immunostimulating composition of claim 15, wherein the antigen is pre-encapsulated into a conformationally stabilizing hydrophilic matrix comprising an appropriate mono, di- or tri-saccharide or other carbohydrate substance by lyophilization prior to its final encapsulation into the PLG microsphere.

Claim 19. The immunostimulating composition of claim 18, wherein the encapsulating microspheres are produced by a solvent extraction process.

Claim 20. The immunostimulating composition of claim 19, wherein said solvent extraction process employs acetonitrile as the polymer solvent, mineral oil as the emulsion's external phase, and heptane as the extractant.

Claim 21. The immunostimulating composition of claim 15, wherein said microspheres further comprise a pharmaceutically acceptable adjuvant.

Claim 22. The immunostimulating composition of claim 15, wherein a molecular weight of the poly(DL-lactide-co-glycolide) is 4,000 to 100,000 daltons.

Claim 23. The immunostimulating composition of claim 15, wherein the relative ratio between the amount of the lactide:glycolide components of the matrix is within the range of 52:48 to 0:100.

Claim 24. The immunostimulating composition of claim 15, wherein the immunogenic substance is a native (oligomeric)HIV-1 envelope antigen.

Claim 25. The immunostimulating composition of claim 15, wherein the amount of said immunogenic substance with in the microsphere comprises between 0.5% to 5.0% of the weight of said composition.

Claim 26. The immunostimulating composition of claim 15, wherein the diameter size range of the microspheres is between 0.1 - 20μm.

Claim 27. The immunostimulating composition of claim 15, wherein the size of more than 50% (by volume) of the vaccine microspheres is between 5 and 10 μm in diameter.

Claim 28. The immunostimulating composition of claim 15, wherein said immunostimulating composition is administered as a mucosal vaccine or a parenteral vaccine.

Claim 29. The immunostimulating composition of claim 28 in the form of a mucosal administerable vaccine wherein the diameter size range of the vaccine microspheres is between 5-10μm.

Claim 30. The immunostimulating composition of claim 28 in the form of a parenteral administerable vaccine wherein the diameter size range of the vaccine microspheres is between 0.1 - 20μm.

Claim 31. The immunostimulating composition of claim 30, wherein said immunogenic substance is present in an amount of 0.5 - 5% antigen by weight.

Claim 32. The immunostimulating composition of claim 15, wherein said bulk matrix encapsulates said immunogenic substance protectively and/or facilitates its interaction with the host immune system to augment its immunogenicity.

Claim 33. A vaccine comprising the immunostimulating composition of claim 15.

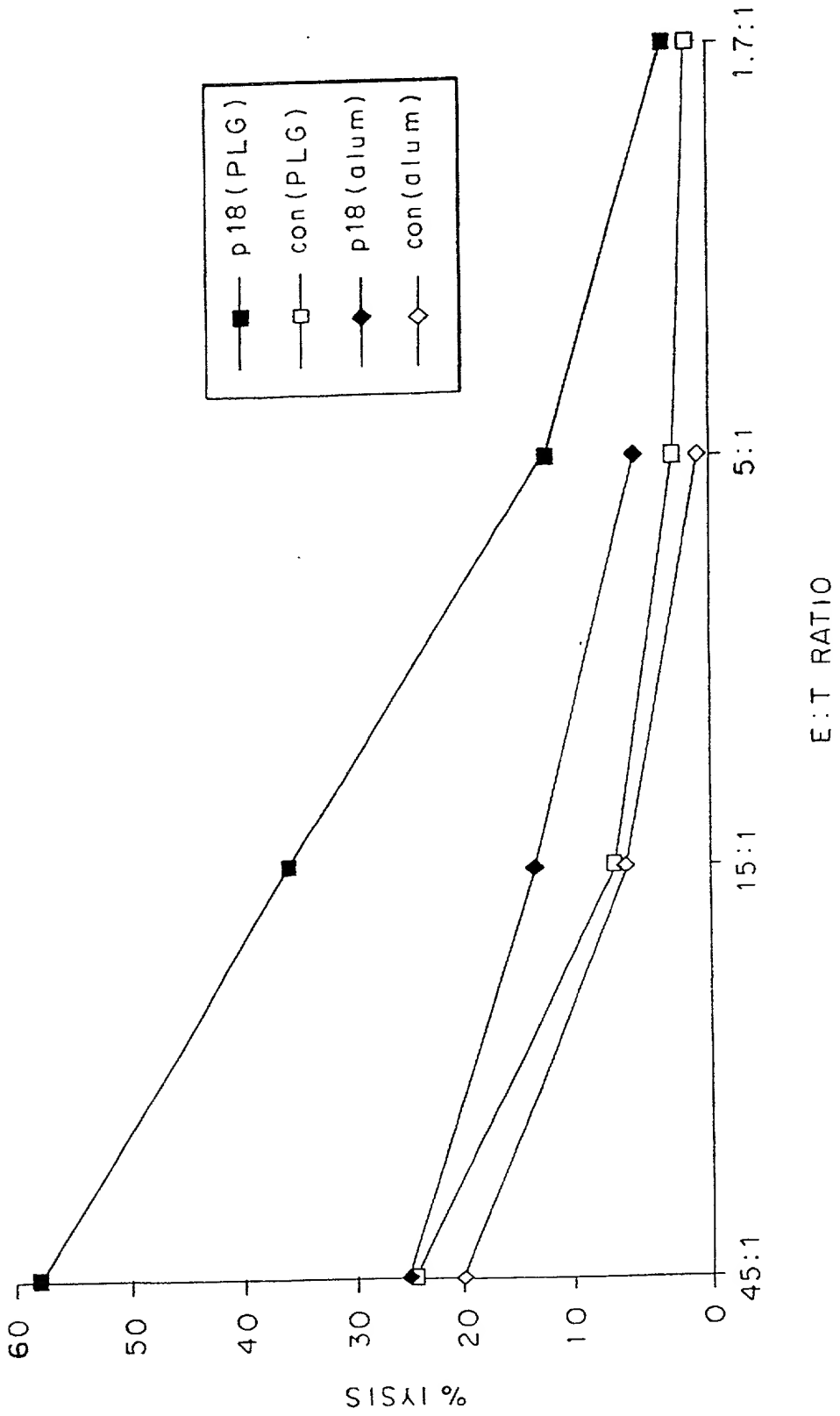
p18(PLG)
con(PLG)
p18(alum)
con(alum)
% LYSIS
60
50
40
30
20
10
0
E:T RATIO
1.7:1
5:1
15:1
45:1

FIG. 1

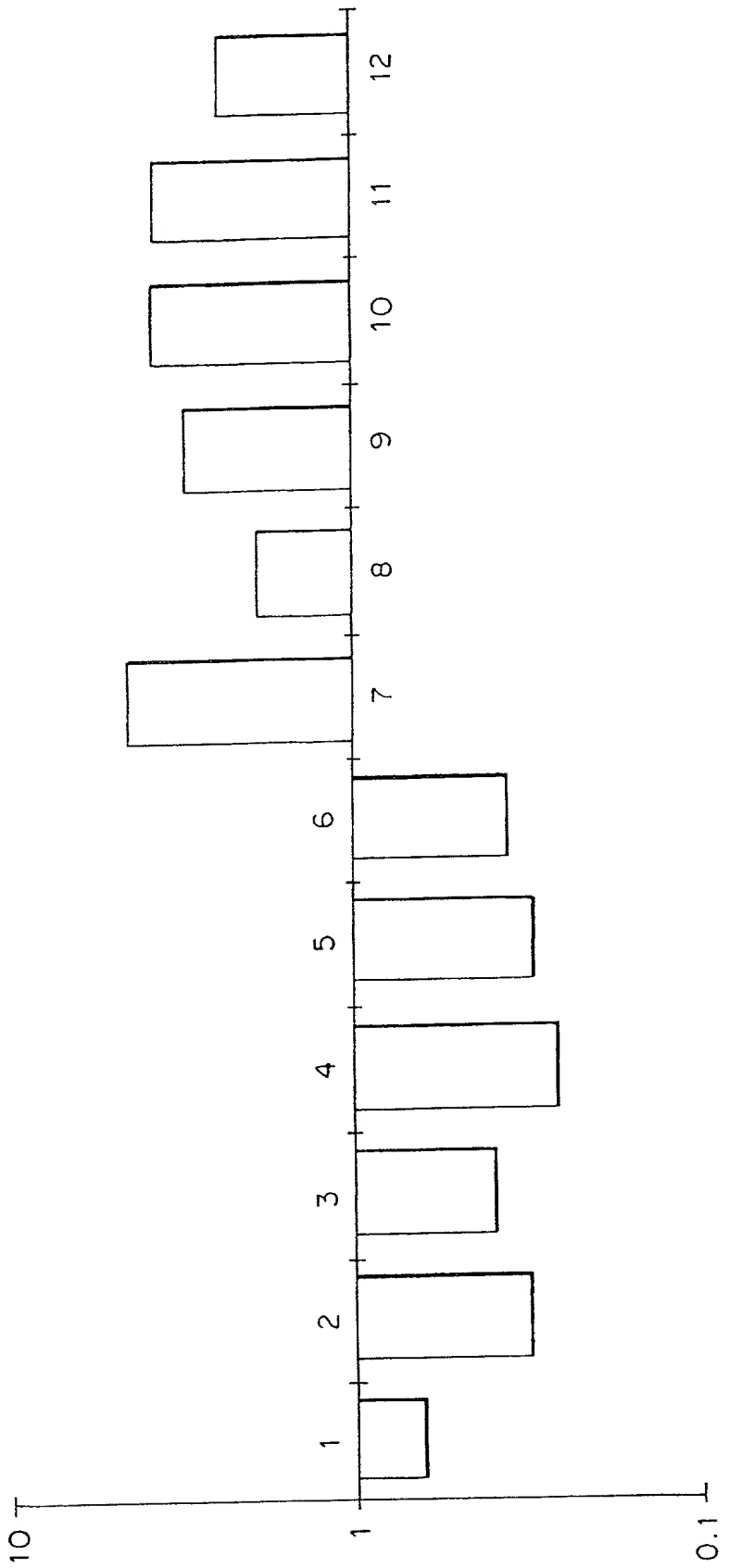

FIG. 2

DECLARATION AND POWER OF ATTORNEY FOR REISSUE APPLICATION

We, Paul R. Burnett, John E. van Hamont, Robert H. Reid, Jean A. Setterstrom, Thomas C. Van Cott, and Deborah Birx hereby declare that we are citizens of the United States and that our residences are as stated below next to our names. We believe that we are the original joint inventors of the invention entitled VACCINES AGAINST INTRACELLULAR PATHOGENS USING ANTIGENS ENCAPSULATED WITHIN BIODEGRADABLE-BIOCOMPATIBLE MICROSPHERES described and claimed in our original application No. 08/598,874 filed February 9, 1996, and the resulting United States Patent No. 5,762,965 which issued June 9, 1998 ("the '965 patent"), for which priority was claimed. The "965 patent" is a continuation in part of Ser. No. 242,960, filed May 16, 1994 and Ser. No. 08/446,149, filed May 22, 1995, which is a continuation of Ser. No 590,308, filed March 16, 1984, abandoned, said Ser. No. 242,960, is a continuation-in-part of Ser. No. 867,301, files April 10, 1992, Patent No. 5,417,986, which is a continuation-in-part of Ser. No. 805,721, filed November 21, 1991, abandoned, which is a continuation-in-part of Ser. No. 690,485, filed April 24, 1991, abandoned, which is a continuation-in-part of Ser. No. 521,945, filed May 11, 1990, abandoned and for which invention a reissue patent is solicited.

We do not know and do not believe that the invention of the '965 patent' was ever known or used in the United States before our invention thereof. Furthermore, we do not know and do not believe that the invention was patented or described in any publication in any country before our invention thereof, or more than one year prior to the original application. We do not know and

do not believe that the invention was in public use or on sale in the United States more than one year prior to the original application. To the best of our knowledge and belief, this invention has not been patented or made the subject of an inventors' certificate in any country foreign to the United States prior to the date of the original application on an application filed by us or our legal representatives or assigns more than 12 months before our original application.

We have reviewed and understand the contents of the attached specification, including the claims, as amended by the amendment of claim 7, 11, 13, the addition of new claims 15-33 and the specification as amended by underlining additions and bracketing deletions. We acknowledge the duty to disclose information of which we are aware and which is material to the examination of the application in accordance with 37 C.F.R. §§ 1.56(a) and 1.175(a) (7).

We believe that through error, without any deceptive intent, the '965 patent is partially inoperative or invalid by reason of the patentee claiming less than the patentee had the right to claim in the patent and the presence of improper multiple dependent claims. In particular, there is a possible defect in that the patentees claimed less than patentees had the right to claim in the patent. Further, claims 7, 11 and 13 appear to be in improper multiple dependent form.

These possible errors arose without deceptive intent during prosecution of the application before the United States Patent and Trademark Office. Upon reviewing the issued claims we realized that the claims did not cover all of the subject matter that the we believe we are entitled to and that claims 7, 11 and 13 are in improper multiple dependant form.

Therefore, by reason of the above-described error, Applicants believe the original patent to be partly inoperative or invalid by reason of the patentees claiming less than patentees had the

right to claim in the patent and the claims are possibly not broad enough to cover all aspects of the invention disclosed in the patent. The possible invalidity of the patent resulted from a failure by ourselves, the assignee and counsel to realize the totality of the subject matter that should have been claimed. By this reissue application, the identified errors are believed to be corrected.

All errors which are being corrected in the present reissue application up to the time of filing of this declaration arose without any deceptive intention on the part of the applicants.

Wherefore we request that we may be allowed to surrender, and we hereby offer to surrender, said U.S. Letters Patent No. 5,762,965 and request that Letters Patent be reissued to ourselves and the assignee, The United States of America as represented by the Secretary of the Army, for the same invention upon the foregoing amended reissue application.

We hereby declare further that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true. We further declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

We hereby appoint Elizabeth Arwine, (Reg. No. 45,867), of the U.S. Army Medical Research and Materiel Command, 504 Scott Street, ATTN: MCMR-JA, Fort Detrick, MD 22702 and Caroline Nash (Reg. No. 36,329) and Marlana K. Titus (Reg. No. 35,843) of Nash & Titus, LLC, 3415 Brookeville Road, Suite 1000, Brookeville, Maryland 20833, (301) 924-9500 or (301) 924-9600 (all communications are to be directed to Nash & Titus, LLC) individually and

collectively as our attorneys to prosecute this application and to transact all business in the Patent and Trademark office connected therewith and with the resultant Patent.

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Date Signed: 12 May 00

EXPRESS MAIL NO. EK044771881US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING REISSUE PATENT APPLICATION

In re Reissue Application of
Burnett et al.

U.S. Patent No. 5,762,965 — Group Art Unit: unknown

Reissue Serial No.: unknown — Examiner: unknown

Reissue Filed: June 2, 2000

FOR: VACCINES AGAINST INTRACELLULAR PATHOGENS USING ANTIGENS ENCAPSULATED WITHIN BIODEGRADABLE-BIOCOMPATIBLE MICROSPHERES

The Commissioner of Patents and Trademarks
Washington, D.C. 20231

NOTICE OF CORRESPONDENCE ADDRESS

Sir:

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Respectfully submitted,

June 2, 2000

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